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Short communication

Ibogaine blocked methamphetamine-induced hyperthermia and induction of heat shock protein in mice

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Abstract

Body temperature changes and heat shock protein (HSP-72) induction in the caudate nucleus were studied in female C57BL/6N mice pretreated with ibogaine (50 mg/kg) and sacrificed 48 h. after a single dose of methamphetamine (20 mg/kg). Methamphetamine injection resulted in hyperthermia and induced HSP-72 expression, whereas treatment with ibogaine alone produced hypothermia. The ibogaine followed by methamphetamine injection showed no hyperthermia and decreased HSP-72 expression. These data indicate that pretreatment with ibogaine can completely block methamphetamine-induced hyperthermia and HSP-72 expression in the striatum. © 1999 Elsevier Science B.V. All rights reserved.

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Ibogaine (IBO) is a naturally occurring alkaloid derived from the root of the African shrub, *Tabernanthe iboga*. Recent evidence suggests that IBO interrupts the physiological and psychological aspects of drug addiction in experimental animals [10]. Based on these findings, IBO is under consideration for the treatment of drug addictions. It has been reported that, in rats, IBO decreases intravenous morphine self-administration [9] and blocks the increase in limbic and striatal dopamine (DA) release [16], as well as the increase in motor activity [17], induced by a low dose of morphine. In mice, IBO reduced cocaine consumption in a drinking preference model [21]. The mechanism by which IBO inhibits the effects of cocaine, morphine and other drugs of abuse is unclear. In an effort to gain further insights into IBO's mode of action, we examined IBO's interaction with methamphetamine, a stimulant known to interact with the DA system.

Methamphetamine (METH) is a drug of abuse known to produce neurotoxic effects in rodents and non-human primates. It has been shown to deplete concentrations of DA

and its metabolites (DOPAC and HVA) [1], and decrease the number of high-affinity DA uptake sites [13] and the activity of tyrosine hydroxylase, the rate-limiting enzyme of DA synthesis [6]. METH has also been reported to induce hyperthermia in rats [4] and mice [2,18]. There are reports, which suggest that this hyperthermia may be the cause of the induction of the 72-kDa heat-shock protein (HSP-72). This contention is supported by the finding that: (1) HSP-72 is not induced if the hyperthermia following METH treatment is prevented [11]; and (2) exposure to a heat stress comparable to that caused by METH will induce the same amount of HSP-72 [19].

HSP are biomolecules that are synthesized in both prokaryotic and eukaryotic cells in response to a variety of stressors, including heavy metals, ischemia, certain disease states, and heat stress [8]. They are believed to function in the assembly and disassembly of protein and protein-containing substances and, in particular, assist protein in assuming their proper three-dimensional confirmation [12]. HSP-72 is the primary inducible form of HSP in mammals and is found only in stressed cells [20]. This makes HSP-72 an excellent marker for cellular stress. Recently, we reported that METH-induced dopaminergic neurotoxicity in mice may be modulated by hyperthermia and HSP-72 expression. A single injection of METH in mice induced hyperthermia and HSP-72 expression [15] and a low envi-

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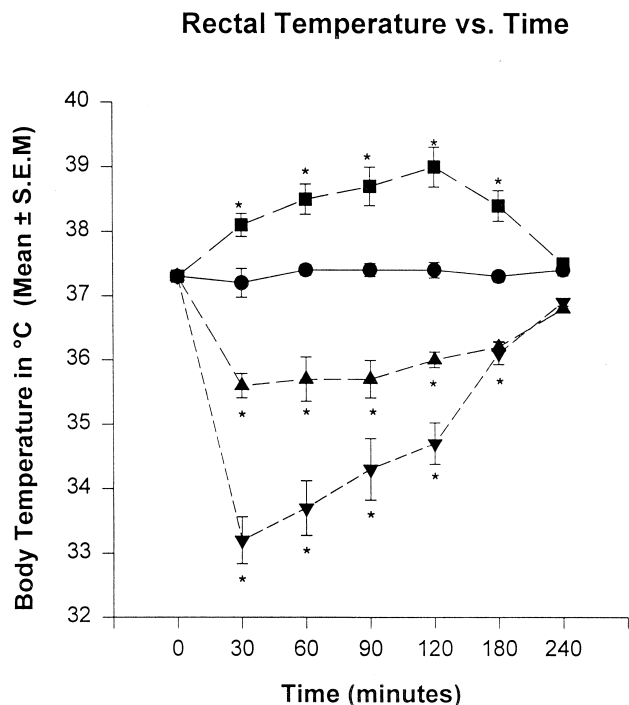


Fig. 1. Time course of the body temperature effects of female C57BL/6N mice at 18 months of age dosed either with saline (●), METH (■) (1×20 mg/kg, i.p.), IBO (▼) (1×50 mg/kg, i.p.) or pretreatment with IBO (1×50 mg/kg, i.p.) followed with METH (▲) (1×20 mg/kg, i.p.). Each value is the mean $\pm$ S.E.M. derived from 10 animals/time point. * Value is statistically different from the respective control ($P < 0.01$).

ronmental temperature and compounds which cause hypothermia, such as MK-801, diazepam and phenobarbital, facilitated the blockade of METH induced striatal dopamine depletion [1]. The present study was designed to determine whether IBO would have any effects on the body temperature changes and HSP-72 expression in the striatum induced by METH.

Eighteen months old female C57BL/6N mice from the NCTR breeding colony were used in this study. Animals were housed 3 per cage under controlled conditions, with food and water available ad libitum. The mice were treated with intraperitoneal (i.p.) Saline ($n = 10$), 50 mg/kg IBO ($n = 10$), 20 mg/kg METH ($n = 10$) or IBO + METH ($n = 10$). Body temperature was recorded by a rectal probe (model 511, YSI, Marion, OH) and a thermistor thermometer (model 5830R, Digitec, Marion, OH) up to 4 h after drug administration. Forty-eight hours after injection, animals were sacrificed and the brains were removed. The striatum was dissected and stored at -70°C until the determination of heat shock protein by immunoblotting. Brain tissue used for the detection of HSP-72 was processed in a similar manner to that previously described [7]. In brief, the tissue was weighed and diluted with 10 volumes of 10 mM Tris buffer (pH 7.4). The tissue was then disrupted by ultrasonication. Protein concentration was determined using the bicinchoninic acid method. The

samples were diluted 1:1 with buffer (125 mM Tris-HCl, 4% sodium dodecylsulfate (SDS), 20% glycerol, 10% 2-mercaptoethanol, pH 6.8). Equal amounts of protein (30 μg) were loaded onto a 10% SDS-PAGE gel, and separated by the Laemmli method using a Mini-protein II series electrophoresis unit (Bio Rad, Hercules, CA). After the SDS-PAGE was performed, the gel was allowed to equilibrate for 5 min in transfer buffer (25 mM Tris, 200 mM glycine, 20% methanol). The proteins were then transferred electrophoretically at 750 mA for 90 min to a $0.45\text{-}\mu\text{m}$ nitrocellulose membrane (Schleicher and Schuell, Keene, NH), using a TE 52X transfer electrophoresis unit (Hoofer Scientific Instruments, San Francisco, CA). After transfer, the membranes were allowed to air dry before being blocked overnight with 3% non-fat dried milk in PBS-T buffer (1.5 mM NaH_2PO_4 , 4.3 mM Na_2HPO_4 , 145 mM NaCl, 0.05% Tween-20). The proteins of interest were detected using anti-HSP-72 (StressGen Biotechnologies, Victoria, B.C.) mouse monoclonal antibody at a dilution of 1:1000. Visualization was achieved with a peroxidase-conjugated anti-mouse IgG antibody (Sigma) and the Renaissance™ Western Blot Chemiluminescence reagent (Dupont, Boston, MA). The blots were scanned and analyzed using a computer program called Scanalytics (Scanalytics, Billerica, MA). Temperature data were evaluated using a one way analysis of variance (ANOVA), followed by a Dunnett's t -test with a Bonferroni correction. A value of $P < 0.05$ was considered to be statistically significant.

Induction of HSP-72 in the Striatum

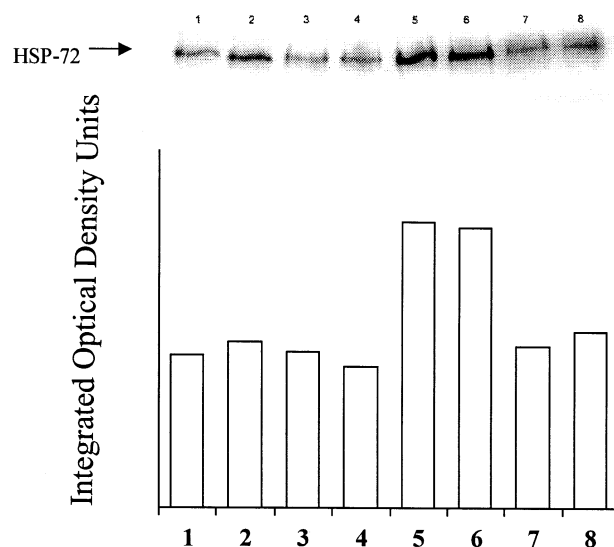


Fig. 2. Immunoblot determination of striatal HSP-72 expression with Scanalytics analysis plotted below. Proteins from three mice striata were pooled and equal amounts of protein were loaded in two lanes for each group. Lanes 1 and 2, saline-treated mice; Lanes 3 and 4, IBO-treated (50 mg/kg) mice; Lanes 5 and 6, METH-treated (20 mg/kg) mice; Lanes 7 and 8, IBO + METH-treated mice.

A single injection of METH (20 mg/kg, i.p.) produced a significant hyperthermia in mice (Fig. 1). The average body temperature of control mice was $37.3 \pm 0.03^\circ\text{C}$. A maximum temperature of $39.0 \pm 0.31^\circ\text{C}$ was recorded 2 h after METH treatment. Temperatures of the METH-treated mice returned to control level 4 h after injection. The injection of mice with 50 mg/kg of IBO produced a significant hypothermia (Fig. 1). A minimum temperature of $33.2 \pm 0.36^\circ\text{C}$ was recorded 0.5 h after IBO treatment. The pretreatment of mice with 50 mg/kg IBO followed by 20 mg/kg METH injection produced no hyperthermia, but these animals still showed hypothermia compared to the controls (Fig. 1). The western blot data for HSP-72 in the striatum with its analysis by Scanalytics reveal a significant induction (200%) when mice were injected with 20 mg/kg of METH (Fig. 2). Pretreatment of mice with 50 mg/kg IBO before 20 mg/kg METH blocked the induction of HSP-72. IBO alone did not induce HSP-72 (Fig. 2).

The mechanism of monoamine depletion following METH treatment remains unresolved, but recent work from this laboratory has revealed some relevant information. We and others have reported that inhibition of neuronal nitric oxide synthase (nNOS) by 7-nitroindazole blocked the METH-induced dopaminergic neurotoxicity [2,5,13] and nNOS knock-out mice are resistant to METH-induced dopaminergic neurotoxicity [14]. Recently, we have also reported that IBO can block the nNOS activity in various brain regions, including the striatum [3]. These data support the hypotheses that reactive oxygen species, and especially peroxynitrite, play an important role in METH-induced neurotoxicity and that IBO may be mediating its effect by the inhibition of NOS [3].

The brain responds to a variety of insults by expressing heat shock genes, which are 'silent' under normal conditions. The heat shock proteins (HSPs) are so named due to the fact that their synthesis was initially found to be enhanced in response to an increase in temperature [22]. But many other insults other than heat stress have been found to increase the expression of HSPs, including neurotoxins, drugs of abuse, environmental stress, metals, oxidative stress and many other pathophysiological states and non-stressful conditions [8]. In various models, the overexpression or induction of HSPs has been shown to confer resistance toward a variety of insults, including hyperthermia, energy deprivation, oxidative stress and glutamate excitotoxicity [8]. The present study confirms and compliments our earlier findings where we reported that METH induced hyperthermia and HSP-70 expression in mouse striatum [15].

In summary, these data clearly demonstrate that a single injection of METH increased the body temperature and induced HSP-72 and that pretreatment with IBO blocked the hyperthermia and induction of HSP-72. The blockade of METH-induced hyperthermia by IBO may be due to inhibition of nNOS, therefore protecting against the heat induced stress and thus blocking the induction of METH-

induced HSP-72 expression. However, it is uncertain whether this interaction would lead to an increase or decrease in METH self-administration. Further studies are needed to address this question directly.

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